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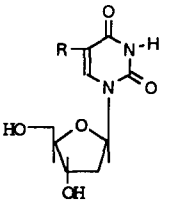
Synthesis and Antiviral Activity of Novel 5-Ethyl- and 5-Vinyl-2'-deoxyuridine Analogues, E. E. Knaus, R. Kumar and L. I. Wiebe. Faculty of Pharmacy, University of Alberta, Edmonton, Alberta, Canada T6G 2N8

2'-Deoxyuridines, that possess a 2-carbon substutuent at C-5, often exhibit potent and selective antiviral activity. A variety of funtionalized C-5 ethyl and vinyl substituents were therefore invesitigated to determine their usefulness as antiviral pharmacophores. The regiospecific addition of halogenoazides (X-N_3) to the 5-vinyl group of **1a** afforded the 5-(1-azido-2-halogenoethyl)- products **1b** (X=Cl,Br,I). Hydrogenation of **1b** (X=I) with Pd/C/H_2 yielded the 5-(1-azidoethyl)- (**1c**) and 5-(1-aminoethyl)- (**1d**) products. The 5-(1-methoxyethyl)-compound (**1e**) was prepared in a similar way. Treatment of **1b** (X=Br) with *t*-BuOK yielded the 5-(1-azidovinyl) derivative (**1f**) which on heating at reflux in toluene gave the azirinyl product (**1g**, 3',5'-di-O-Ac derivative). 5-(1-Aminoethyl)-2'-deoxyuridine (**1d**) was 10-fold more potent than acyclovir against HSV-1 with a S.I. > 50,000. The 5-(1-azido-2-chloroethyl)- compound (**1b**,X=Cl) was equipotent to acyclovir for HSV-1, VZV and EBV.



1a, R= -CH=CH_2
1b, R= -CH(N_3)CH_2X
1c, R= -CH(N_3)CH_3
1d, R= -CH(NH_2)CH_3

1e, R= -CH(OMe)CH_3
1f, R= -C(N_3)=CH_2
1g, R= 

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Preliminary Characterization of HSV-1 Protease by Expression in E. coli

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A viral specific protease has been identified in Herpes Simplex Virus-1 as the UL26 gene product. The HSV-1 protease is responsible for the C-terminal cleavage of the nucleocapsid associated proteins, ICP35 c and d, to their post-translationally modified counterparts, ICP35 e and f. A temperature sensitive mutation in the UL26 gene affects the processing of ICP35, and results in the failure of nucleocapsids to package DNA. This mutation suggests that HSV-1 protease cleavage of ICP35 is essential for production of infectious virions. To further evaluate HSV protease as a potential target for anti-Herpes therapy, the UL26 gene product was expressed and characterized in E. coli. The HSV-1 protease and its substrate are 3' co-terminal, withthe entire amino acid sequence of ICP35 contained within the protease. Self-processing of the protease is therefore expected at the C-terminus. Auto-proteolytic activity of the E. coli expressed HSV-1 protease was demonstrated by pulse chase and Western analysis. To exclude the possibility that bacterial proteases were responsible for the observed processing, an inactive point mutation of HSV-1 protease was expressed in E. coli. This mutant displayed no auto-proteolytic processing. Coexpression of the inactive mutant with active HSV-1 protease resulted in processing of the mutant. This bacterial trans-assay will allow detailed structure-function analysis of the HSV-1 protease and provide a biological assay to study substrate cleavage specificity.